Egypt. J. Plant Prot. Res. Inst. (2019), 2 (1): 165- 175

Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Relationship between enzyme activity and resistance to insecticides in the tested field strains of *Aphis gossypii* (Hemipetra: Aphididae)

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ARTICLE INFO

Article History
Received: 23 /1 / 2019
Accepted: 26 / 2 /2019

Keywords
Aphis gossypii, resistance, *α*- and *β*-esterase and acetylcholinesterase (AChE).

Abstract:

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is phloem feeders, often found on the abaxial leaf surface and excrete the excess material obtained from the phloem as honeydew and in high numbers can cause leaf curling and its honeydew can create a shine on the leaves. Resistance to several insecticides belonging to different groups in the six field strains of the cotton aphid, *A. gossypii* collected from Behera, Dakahlia, Menofia, Skarkia, Gharbia and Beni-Suif Governorates in 2010 cotton season was investigated using slide-dipping method. The insecticides used in this study belong to organophosphates, carbamates, pyrethorids and neonicotinoids. The results indicated that the field strains exhibited high levels of resistance to the organophosphates fenitrothion, prothiofos, pirimiphos methyl and malathion (RR=9.6-49-fold), the carbamates pirimicarb and carbosulfan (RR= 14.8-53.4-fold), pyrethroids lambda-cyhalothrin, alpha-cypermethrin, fenpropathrin, and es-fenvalerate (RR=11.9-44.7-fold) and the neonicotinoids imidacloprid, acetamiprid and thiamethoxam (RR = 2.1-8.2-fold), except for thiamethoxam in Menofia and Dakahlia (11.5 and 14.0-fold, respectively). Enzyme activities of esterases and acetylcholinesterase (AChE) were studied in six field strains of *A. gossypii*. The results indicated that field strains exhibited higher levels of *α* - and *β* - esterase activities and lower AChE activity than those achieved in the susceptible strain. These results indicated that esterases may appear to play insecticide resistance belonging to different groups, while AChE may also appear to play a role in organophosphates and carbamates. However, positive correlation between esterase activity and insecticide resistance or negative correlation between AChE and organophosphate or carbamate resistance but not significant was observed at 1% or 5% accuracy level. Therefore, esterase and AChE activities may not relate to insecticides resistance.

Introduction

The cotton aphid, *Aphis gossypii* Glover (Hemipetra: Aphididae) is an economically important pest in cotton fields in Egypt as well as many other countries. One of the commonly practiced approaches utilized by growers to protect cotton plants from aphids is the use of chemical insecticides. As such applications are frequent, the role of most of the abundant natural enemies is eliminated particularly after the aphid develop resistance to these insecticides (Godfrey *et al*.,2001), thus making subsequent treatments inefficient and leading to an increase in aphid population levels (Godfrey and Fuson, 2009). In Egypt, several organophosphorus and carbamate insecticides had been used against cotton aphids since 1970 and until 2000. The carbamate carbosulfan and neonicotinoids had been also used against cotton aphids since and until now. Resistance to organophosphorus, carbamate and pyrethroid insecticides had been reported by several authors in many countries (Li *et al*., 2003; Ahmad *et al*., 2003; Herron and Wilson, 2004; Jhansi and Subbaratnam, 2005 a, b and Singab, 2007a, b). Resistance in cotton aphids to neonicotinoids had been reported by Wang *et al*. (2001, 2002); Yu *et al*. (2004); Singab (2007b) and Barakat *et al*. (2013). One of the cotton aphids, *A. gossypii* and resistance to organophosphorus, carbamate and pyrethroid insecticides was found to associate with high esterase and oxidase activities (Xie *et al*., 2002 and Ezz el Din, 2003). Insensitivity of acetylcholinesterase (AChE) was also found for OP and carbamate resistance (Sun *et al*., 2002; Xie *et al*., 2002; Benting and Nauen, 2004; Andrew *et al*., 2004 and Toda *et al*., 2004). As for neonicotinoid resistance, the activities of carboxyl esterase and glutathione-s transferases were main metabolic mechanism of resistance (Pan *et al*., 2003).

The present work presents focuses on survey of the resistance to the insecticides commonly used in Egypt for the control of the cotton aphis, *A. gossypii* field populations collected from six Governorates in 2010 cotton growing season to determine the levels of resistance of certain tested insecticides. Biochemical determinations were also studied. Enzyme activities of esterases and acetylcholinestrase were also investegated in an attempt to clarify the correlation between resistance development and the activity of these enzymes.

Materials and methods

1. Monitoring of resistance to insecticides in the field strains of *Aphis gossypii* during 2010 cotton growing season:

1.1. The laboratory strain of the cotton aphid, *Aphis gossypii* :

The susceptible strain of *A. gossypii* was obtained from a cotton field population at Fayoum Governorate then reared entirely unexposed to any insecticides at the Central Agricultural Pesticides Laboratory for ten generations under constant conditions of 27± 2º C, 55 ± 5 % RH. This strain was used for all bioassay investigations and regarded as a reference strain in studies on monitoring resistance and biochemical determinations.

1.2. The field strains of the cotton aphid, *Aphis gossypii* :

Field strains of *A. gossypii* were collected from selected cotton fields at Behera, Dakahlia, Menofia, Skarkia, Gharbia and Beni-Suif Governorates in 2010 befor the commencement of spray season early (May).

Bioassay of the tested insecticides against the cotton aphid, *Aphis gossypii*: Slide-dipping technique (Dittrich, 1962) was used to obtain concentration mortality lines of the tested insecticides against the adult stage of *A. gossypii*. Five different concentrations of each tested insecticide were prepared by dilution in water. By

means of a fine brush, ten adult aphids were affixed to a piece double face scotch tap then stuck tightly to glass a slide on the dorsal part. Slides were then dipped in the prepared insecticide aqueous solutions for ten seconds, three replicates were used for each concentration, mortality counts were recorded 2 hours after treatment and the percentages of mortality were corrected according to Abbott's formula (1925) and mortality data were subjected to statistical analysis as described by Busvine (1957). The rates of resistance were expressed as resistance ratios (RR) at LC_{50} value of the field strains as compared with the LC_{50} value of the laboratory strain.

Resistance ratio (RR) = LC_{50} of the field strains / LC_{50} of the laboratory strain

1.4. Insecticide used:

1.4.1. Organophosphates: chlorpyrifos methyl (Reldan, 50 % EC), chlorpyrifos ethyl (Dursban, 5% EC), pirimiphos methyl (Actellic 50 % EC), profenofos (Selecron, 50% EC), malathion (Malason, 57% EC), prothiofos (Tokuthion, 5% EC), fenitrothion (Sumithion, 57% EC)

1.4.2. Carbamates: (carbosulfan (Marshal, 50% WG), methomyl (Lannate, 90% SC) and pirimicarb (Aphox**,** 50% WG)

1.4.3. Pyrethroid: deltamethrin (Decis, 2.5% EC), fenpropathrin (Fenithrin, 30 % EC), esfenvalerate (Sumi-alfa, 5% EC), alpha-cypermethrin (Alfa-cyper, 10% EC), lambda-cyhalothrin (Karate, 2.5% EC).

1.4.4. Neonicotinoids: imidacloprid (Confidor, 20% SL), Thiamethoxam (Actara, 25% WG), acetamiprid (Mospilan 20% WP).

2. Enzyme assay:

2.1. Preparation of haemogenate samples for biochemical analysis:

Adult indiriduals of *A. gosypii* were homogenized in distilled water at 500 rpm using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were collected (on ice according to Liu *et al.*, 2010) in cold tubes previously coated with crystals of phenylthiourea to prevent melanization, then centrifuged at 6000 rpm for 10 min at 5°C. using a beckman gs-6r centrifuge. After centrifugation, the supernatant fluid was divided into small aliquots of 0.5 ml each and stored at -20 °C. until needed. Three replicates were made for every biochemical determination.

2.2. Determination of acetylcholinesterase activity:

The activity of acetylcholinesterase (AChE) was measured according to the method described by Simpson *et al.* (1964) using acetylcholine bromide (AChBr) as a substrate at a level of 6×10^{-3} *M* Test tubes (T) contains 0.2 ml of adult tissues homogenate, 0.5 ml 0.067 M phosphate buffer and 0.5 ml substrate (3 mM AChBr) were prepared. The total substrate tubes (TS) contained 0.7 ml phosphate buffer (0,067 mM) and 0.5 ml substrate. A control tube (C) contains 0.2 ml of adult homogenate and 1 ml of phosphate buffer was prepared. All test tubes were incubated for exactly 30 minutes at 37°C. After incubation period, 1 ml of alkaline hydroxylamine (equal to a volume of 2 *M* hydroxylamine chloride and 3.5 *M* NaOH mixed thoroughly shortly before use was added to all tubes. Tubes were shaken well and allowed to stand for 2 minutes then 0.5 ml of HCl (1 part of conc. HCl mixed with 2 parts of distilled water) was added. The mixture was shaken vigorously then allowed to stand for 2 minutes 0.5 ml of ferric chloride solution (0.92 *M* FeCl3 in 0.1 *M* HCl) was added to all test tubes and mixed well. The resulting mixture was centrifuged and the supernatant was measured spectrophotometrically at 515 nm. The activity of the homogenate was calculated by applying the following equation:

(TS+C) – T / Weight/ml homogenate/30 = µg substrate hydrolyzed/min/g body weight. Where:(T) = test, (TS) = substrate, (C) = control

2.3. Determination of non-specific esterases activities:

Alpha-and beta-esterases (α-E, β-E, respectively) were determined according to the method of Van Asperen (1962) using α-naphthyl acetate and β-naphthyl acetate as substrates. Naphthol produced as a result of substrate hydrolysis was measured by addition of diazoblue sodium lauryl sulphate solution which produces a strong blue colour in the case of α-naphthol or a strong red colour that of β-naphthol. The resulting colour was measured spectrophotometrically using a Milton Roy Spectronic (model 1201) spectrophotometer. The reaction mixture consisted of 5 ml substrate solution ($3x10^{-4}$*M* α-orβ-naphthyl acetate, 1% acetone and 0.04 *M* phosphate buffer pH7) added to 20 µl of adult homogenate. The mixture was incubated for exactly 15 minutes at 27 °C. then 1 ml of diazoblue color reagent (prepared by mixing 2 parts of 1% diazoblue B and 5 parts of 5% sodium lauryl sulphate) was added. The developed color was measured at 600 and 555 nm for α- and β-naphthol, respectively. Enzyme activity was expressed as µg α- or β-naphthol released / min. / adult.

2.3.1. Preparation of the standard curves of α- and β-naphthol

Stock solutions were prepared by dissolving 20 mg α- or β-naphthol in 100 ml of 0.04 M phosphate buffer (pH 7). Ten milliliters of the stock solution were diluted up to 100 ml by the phosphate buffer. Aliquots containing of 2.5, 5, 10, 20 and 40 µg of α-naphthol or 2.5, 5, 7.5, 10 and 20 µg β-naphthol were pippetted into test tubes and completed to 5 ml by phosphate buffer. One milliliter of diazoblue reagent was added and the developed color was measured as mentioned earlier. The standard curves of both α-E and β-E were blatted by O.D. (Optical Density) against concentration according by Bradford (1976).

3.Determination of total proteins:

Total proteins were determined according to the method described by **Bradford (1976).**

3.1. Preparation of protein reagent:

Coomassie Brilliant Blue G-250 (100 mg) was dissolved in 50 ml 95% ethanol. To this solution, 100 ml of 85% (w/v) phosphoric acid were added. The resulting solution was diluted to a final volume of 1 liter.

3.2. Protein assay:

- Sample solutions of 50 µl were pipetted into a test tubes and the volume adjusted to 0.1 ml with phosphate buffer (pH 6.6).
- 5 ml of protein reagent was added to every test tube and the contents were the roughly mixed (inversion or overtaxing).
- Absorbance at 595 nm was measured after 2 min. then before 1 hr against blank prepared from 0.1 ml phosphate buffer (pH 6.6) and 5 ml protein reagent. The weight of protein was plotted against the corresponding absorbance thus resulting in standard curve used to determine the protein in the unknown samples.

3.3. Preparation of standard curve of protein:

For the preparation of the standard curve of protein serial concentrations of Bovine serum albumin solutions containing 10 to 100 µg of protein were pipetted into test tubes and the volume was adjusted to 0.1 ml with phosphate buffer (pH 6.6). Five ml of protein reagent were added and the resulting colour was measured spectrophotometrically at 595 nm as mentioned before. The optical densities were plotted against concentrations to construct. The standard curve of protein was then plotted by O.D. (Optical Density) against concentration.

Results and discussion

1. **Monitoring of resistance of tested insecticides in the field strains of *Aphis gossypii* in 2010 cotton growing season**

The levels of resistance to organophosphorus, carbamate, and pyrethroid and neonicotinoid groups of

insecticides against the 6 tested field strains of *A. gossypii* collected from six governorates are shown in Table (1). Most of the field strains exhibited high levels of resistance to the organophosphorus fenitrothion, prothiofos, pirimiphos methyl and malathion (RR=9.6-49-fold), the carbamates pirimicarb and carbosulfan (RR= 14.8-53.4-fold) and the pyrethroids lambda-cyhalothrin, alpha-cypermethrin, fenpropathrin, and es-fenvalerate (RR=11.9-44.7-fold). On the contrary for malathion in Gharbia and Behera (RR=6.6 and 7.4-fold), carbosulfan in Behera and Dakahlia (RR=1.6 and 3.6-fold), es-fenvalerate in Behera and Dakahlia (RR=4.4 and 5.6-fold), showed low to moderate levels of resistance to the organophosphorus chlorpyrifos methyl, chlorpyrifos ethyl and profenofos (RR= 1.7-7.7-fold), the carbamate methomyl (RR = 2.2-5.3-fold), the pyrethroid deltamethrin (RR = 2.5-9.0-fold) and the neonicotinoids imidacloprid, acetamiprid and thiamethoxam (RR = 2.1-8.2-fold), except for chlorpyrifos ethyl in Sharkia and Menofia (RR=11.1 and 13.5-fold, respectively), thiamethoxam in Menofia and Dakahlia (11.5 and 14.0-fold, respectively).

Table (1): Resistance ratioes of insecticides in six field strains of *Aphis gossypii* collected from different Governorates in 2010 cotton growing season

Group	Insecticide	LC_{50}ppm S-strain	Resistance ration (RR*)					
			Behera	Dakahlia	Menofia	Gharbia	Sharkia	Beni-Suef
Organophos-phorus	Chlorpyrifos methyl (Reldan)	63.8	7	4.2	1.7	3.6	5	6.5
	Chlorpyrifos ethyl (Dursban)	26.32	5.3	6.3	13.5	5	11.5	2
	Pirimiphos methyl (Actellic)	70.5	24.8	15.4	36.2	19.2	31.5	9.6
	Profenofos (Selecron)	208.6	2.5	7.7	4.3	4.3	7.2	2.6
	Malathion (Malason)	149.3	7.4	10.7	23.1	6.6	20.8	17.3
	Prothiofos (Tokuthion)	154.49	16	12.3	24.5	19	31.4	14.2
	Fenitrothion (Sumithion)	111.67	40.2	17.9	49	32.1	56.3	25.3
Carbamates	Carbosulfan (Marshal)	17.66	1.6	3.6	13.5	14.8	44.1	53.4
	Methomyl (Lannate)	41.25	2.2	5.3	4.1	2.8	5.1	---
	Pirimicarb (Aphox)	189.04	39.9	17.1	50.4	45.6	43.2	17.5
Pyrethroids	Deltamethrin (Decis)	2.22	2.5	3.9	9	4.2	8.4	7.3
	Lambda-cyhalothrin (Karate)	5.89	11.9	44.7	15.4	16.3	36.1	32.6
	Es-fenvalerate (Sumi-alpha)	4.04	4.4	5.6	15.4	9.2	27.1	23.5
	Fenpropathrin (Fenethrin)	12.99	13.9	22.9	21.8	11.2	18.6	27.9
	Alpha-cypermethrin (Alpha-cyper)	8.94	16.7	19.8	12.7	14.4	22.9	26
Neonicotinoids	Imidacloprid (Confidor)	7.35	2.9	2.8	3.7	3.1	4.1	2.1
	Acetamiprid Mospilan	5.61	4.8	3.4	6.3	2.2	5.3	4.2
	Thiamethoxam (Actara)	8.84	6.5	14	11.5	7	8.2	8.1

*RR (Resistance ratio) = LC_{50} of the field strain / LC_{50} of the laboratory strain

2.Determination of acetylcholinestrase and esterase activities in the field strains of *Aphis gossypii*:

The activities of acetylcholinesterase (AChE), *α*-esterase (*α* EST) and β-esterase (β EST) were determined in six field strains of *A. gossypii* collected from six governorates. Data are presented in Table (2).

Table (2): Total protein and specific activity of acetylcholinesterase (AChE), α-esterase and β-esterase in the susceptible strain (s-strain) and 6 field strains of *Aphis gossypii* collected from different Governorates in 2010 cotton growing season.

Strain	Total protein mg/ g b.w.	Specific activity µg /min/g b.w.			Ratio of F/S*			
		AChE	*α*- esterase	*β*- estruses	Total Protein	AChE	*α*- esterase	*β*- esterase
susceptible strain	19.46	1174.6	1126.72	923.87	1	1	1	1
Beni-Suf Strain	23.07	1061.3	1594.01	1257.58	1.19	0.9	1.41	1.36
Menofia Strain	22.99	888.29	1883.27	1199.42	1.18	0.75	1.67	1.3
Dakahlia Strain	25.19	992.87	1636.78	1200.25	1.29	0.84	1.45	1.3
Behera Strain	20.84	1112.4	1468.91	1122.75	1.07	0.94	1.3	1.22
Sharkia Strain	23.76	961.47	2079.37	1368.56	1.22	0.81	1.85	1.48
Gharbia Strain	22.12	992.68	1656.06	1111.42	1.13	0.85	1.47	1.2

* F/S = Activity of enzyme or total protein in the field strain / Activity of enzyme or total protein in the susceptible strain

2.1. Actylcholinesterase (AChE):

As with AChE, the results refer that the activity of AChE in the field strains was low when compared to the susceptible strain. The activity of AChE in the S-strain was 1174.62µg/min/g b.w., while in the field strains ranged 888.29-1112.4 µg/min/g b.w. The lowest level of activity recorded in Menofia strain (0.75 times) followed by Sharkia strain (0.81 times), Dakahlia strain (0.84 times), Gharbia strain (0.85 times), Beni-Suef strain (0.90 times) and Behera strain (0.94 times). Reduced activity of AChE was also reported by Singab (1996) on OP or carbamate- resistant strain of *Spodoptera littoralis* and Rofail *et al*. (1995) on resistant strain of *Pectinophora gossypii*. Several authors contributed to the reduced sensitivity of AChE in the OP or carbamate - resistant strains of *A. gossypii* and its responsibility for resistance to insecticides (Sun *et al*. 1987 and 1994; Suzuki and Hama 1994; Xie *et al*. 2002; Andrews *et al*. 2004 and Benting and Nauen 2004).

2.2. *α*- and *β*- esterases:

For of *α*- EST, data in Table (2) show that all field strains showed a high activity than the susceptible strain (S-strain). The activities of *α*- EST in the different considered field strains of *A. gossypii* collected from different Governorates. For *α*-esterases, (Table 2) all field strains showed a higher activity than the susceptible strain (S-starin). The activity of *α*-esterases in the S-starin was 1126.72 µg/min/g b.w. compared to activities of 1468.91-2079.37 µg/min/g b.w. in field strains. The highest level of activity was recorded in Sharkia strain (1.85 times), followed by Menofia strain (1.67 times) Then Gharbia strain (1.47 times), Dakahlia strain (1.45 times), Beni-Suef strain (1.41 times) and finally Behera strain (1.30 times).

For *β*-EST the same trend of activity was obtained Table (2), all field strains exhibited higher activity than that obtained in S-strain, where the activity of *β*-esterases in S-strain was 923.87 µg / min/g b.w. compared to 1111.42-1368.56µg/min/g b.w. in the field strains. The highest level of resistance was records in Shrike strain (1.48

times) followed by Beni-Suef strain (1.36 times) then Menofia strain (1.30 times), Dakahlia strain (1.30 times), Behera strain (1.22 times) and finally Gharbia strain (1.20 times).

These results indicate that the field strains of *A. gossypii* exhibited relatively higher levels of EST activities than in the S-strain. Thus, they seem to play an important role in determining insecticides resistance in the field strains of considered insect pest. Several authors reported that *A. gossypii* resistance to organophosphorus, carbamate, pyrethroid or neonicotinoid insecticides was associated with high EST activity (Saito, 1990, Sun *et al*. 1994, Xie *et al*. 2002, Ezzel-Din 2003, Pan *et al*. 2003 and Jhansi and Subbaratnam, 2004)

3.The relationship between enzyme activity and resistance to insecticide in the tested field strains of *Aphis gossypii*:

The relationship between enzyme activity and insecticide resistance was studied statistically in six field strain in ***A. gossypii*** collected from six governorates in 2010 cotton growing season. Data obtained are presented in Table (3).

3.1. The relationship between Actylcholinesterase activity and insecticide resistance:

Table (3) shows a negative correlation between AChE activity and the levels of resistance for all tested OP and carbamate insecticides except the OP chlorpyrifos methyl and the carbamate carbosulfan that showed a positive correlation. However, these correlations were not significant at either 1% or 5% accuracy levels (r coefficients = -0.200 and -0.745, respectively, at n=6). Such correlations refer that decrease in AChE activity is not statistically associated with increase in insecticide resistance. In other words, AChE activity is not significantly related to resistance level.

3.2. The relationship between *α*-esterase activity and insecticide resistance:

Results in Table (3) shows a positive correlation between α-esterase activity and the levels of resistance for all tested insecticides belonging to organophosphorus, carbamates, pyrethroids and neonicotinoids insecticides, except the organophosphorus, chlorpyrifos methyl that exhibited a negative correlation coefficient. All calculated correlation coefficient were not significant at either 1% or 5% accuracy levels (r = +0.144 and +0.795, n=6). This mesns that increase in α-esterase activity is not statistically associated with increase in insecticide resistance. In other words, α-esterase activity is not related to insecticide resistance. However, the calculated r values nearly approached the tabular coverponding values, especially for the organophosphorus: chlorpyrifos ethyl (r = +0.795), malathion (r = +0.773), fenitrothion (r = +0.713), pirimiphos methyl (r = +0.666); the carbamate methomyl (r = +0.701); the pyrethroids es-fenvalerate (r = +0.778), deltamethrin (r = +0.765) and the neonicotinoid imidacloprid (r = +0.781). Thus, α-esterase seems to be clear to play a role in the resistance to these compounds.

3.3. The relationship between *β*-esterase activity and insecticide resistance:

Table (3) shows that there is a positive correlation between β-esterase activity and the level of resistance for all tested insecticides belonging to organophosphorus, carbamates, pyrethroids and neonicotinoids except the organophosphorus chlorpyrifos ethyl, pirimiphos methyl, fenitrothion; the carbamate pirimicarb and the neonicotinoid thiamethoxam. All correlation coefficient was significant at either 1% or 5% accuracylevels (r = +0.010 and +0.773, n=6). Such correlation refers that increase in β-esterase activity is not related to with increase in insecticide resistance.

It is concluded that the field populations of *A. gossypii* from different Governorates seem to possess a sort of resistance to most of the tested insecticides representing organophosphorus, carbamates

and pyrethroids. These populations expressed a high esterase activity and less AChE activity than those expressed by the S-strain. However, no statistically significant correlations could be drawn between insecticide resistance and enzyme activities at either 1% or 5% levels of accuracy. This result contradicts with the findingd of Xie *et al*. (2002) who showed that the resistance of *A. gossypii* populations to organophosphate insecticides involves the *α*-esterase activity. The same authors added that the insensitivity of AChE to the carbamate methomyl might be responsible for methomyl resistance.

Contributions to the role of esterase activity and insensitivity of AChE in resistance to organophosphorus and carbamates insecticides as well as esterase activity in resistance to pyrethroids and neonicotinoids insecticides in several pests are given the work of Ghoneim *et al.* (1994) who reported that correlation between esterase activity and resistance to insecticides in *S.littoralis* populations in Egypt was recognized for the pyrethroids deltamethrin, alpha-cypermethrin, fenpropathrin and the organophosphorus cyanophos. Similar results were obtained by Saleh *et al*. (1986) with fenitrothion on a cyanophos-resistant strain of *S. littoralis.* Rofail *et al*. (1995) referred to a correlation between resistance ratio to cyanophos and the high activity of *α*-esterase or low activity of AChE in a cyanophos-resistant strain of *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae).

Table (3): The relationship between insecticide resistance and enzyme activity:

Group	Insecticide	N	Correlation Coefficient (r)		
			AChE	α-esterase	β-esterase
Organophosphorus	Chlorpyrifos methyl (Reldan)	6	0.927	-0.510	0.157
	Chlorpyrifos ethyl (Dursban)	6	-0.745	0.795	-0.095
	Pirimiphos methyl (Actellic)	6	-0.435	0.666	-0.263
	Profenofos (Selecron)	6	-0.576	0.481	0.723
	Malathion (Malason)	6	-0.551	0.773	0.214
	Prothiofos (Tokuthion)	6	-0.425	0.275	0.178
	Fenitrothion (Sumithion)	6	-0.200	0.713	-0.090
Carbamates	Carbosulfan (Marshal)	6	0.110	0.401	0.558
	Methomyl (Lannate)	5	-0.214	0.701	0.762
	Pirimicarb (Aphox)	6	-0.234	0.439	-0.447
Pyrethroids	Deltamethrin (Decis)	6	-----	0.765	0.154
	Lambda-cyhalothrin (Karate)	6	------	0.233	0.773
	Es-fenvalerate (Sumi-alpha)	6	-----	0.778	0.431
	Fenpropathrin (Fenethrin)	6	------	0.105	0.310
	Alpha-cypermethrin (Fastac)	6	-----	0.051	0.301
Neonicotinoids	Imidacloprid (Confidor)	6	------	0.781	0.010
	Acetamiprid (Mospilan)	6	-----	0.387	-0.371
	Thiamethoxam (Acara)	6	------	0.144	0.071
Tabular r	N = 6	5% = 0.811			
		1% = 0.917			
	N = 5	5% = 0.878			
		1% = 0.959			

The statistical analyses for the relationships between resistance ratio and enzyme activity revealed no significant correlations between resistances to any tested insecticides and the activity of any of the considered enzymes at either 1% or 5% level of the accuracy (Table, 3). This indicating that a decrease in AChE activity or increase in esterase activity is not associated with increase in resistance.

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